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Evaluation of allelic alterations in STR in different kinds of tumors and formalyn fixed tissues—possible pitfalls in forensic casework

G. Margiotta*, A. Coletti, M. Lancia, L. Lottanti, E. Carnevali, M. Bacci

Section of Legal Medicine, University of Perugia, Italy

Abstract. Nowadays, the use of formalyn fixed tissue for forensic identification is frequently requested. This is why forensic laboratories must often study normal or tumour tissue specimens that are usually archived with this method. The somatic instability of tumour tissue on STR loci and the DNA damages caused by formaldeide are well described. These conditions can cause an incorrect allelic determination that makes a forensic identification fail. In order to evaluate the real incidence of the genetic alterations caused by somatic instability of tumour tissue, and the incidence of the DNA damages caused by formalyn, we studied 25 specimens of patients who have been operated for neoplasia. For each patient, we studied a specimen of fresh tumour tissue and a specimen of formalyn-fixed tumour tissue, and the results of these analyses were compared to a specimen of fresh normal tissue and to a specimen of formalyn-fixed normal tissue of the same patient. © 2005 Published by Elsevier B.V.

Keywords: Formalyn; Somatic instability; DNA damages

1. Introduction

Analysis of STRs offers an efficient and reliable way of discriminating between sample source and individuals, e.g. a mislabelled histological tissue block could be matched to its source. Neoplastic tissues manifest a great variety of genetic alterations, such as chromosomal instability, allelic deletions (loss of heterozygosity, LOH) and allelic insertions (microsatellite instability, MSI).

* Corresponding author. Tel.: +39 0744 205055; fax: +39 0744 220236.
E-mail address: gabriele.margiotta@poste.it (G. Margiotta).

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Table 1

	Total alteration (%)	pLOH		LOH		Add allele		Subst allele	
		N	%	*N*	%	*N*	%	*N*	%
Normal Fixed Tissues	20	6	50	3	25	3	25	0	0
Tumor Fresh Tissues	64	37	72.6	9	17.6	5	9.8	0	0
Tumor Fixed Tissues	72	31	39.7	35	44.9	11	14.1	1	1.3

In this study, we analysed 25 primary carcinomas. The DNA profiles were achieved by markers and genotyping protocols used in forensic studies. The aim was to evaluate the real incidence of the genetic alterations caused by somatic instability of tumour tissue, and the incidence of the DNA damages by formalyn, to estimate the reliability of the results when neoplastic fixed tissues are used as a source of genetic information.

2. Materials and methods

Surgically resected tissue specimens from sporadic primary cancers and adjacent cancer-free areas were collected from 25 individuals. For each patient, we studied a specimen of fresh tumour tissue and a specimen of formalyn-fixed tumour tissue, and the results of these analyses were compared to a specimen of fresh normal tissue and to a specimen of formalyn-fixed normal tissue of the same patient. The specimens were from colon (15), lugs (2), stomach (6), thyroid (1), and bladder (1).

After excision, a portion of the tumours and the non-cancerous margins were separated and frozen, another portion of the tumours and the non-cancerous margins were kept in formalyn 10%. After 48 h of permanence in formalyn these specimens were rinsed in running water for 10 h and after were kept in etanolo 80%. In these conditions specimens were kept until processing.

Digestion of tissue samples was carried out with ProteinaseK, TNE, Sarkosyl, DTT at 56 °C overnight, followed by purification with 2 passages with Phenol/Chloroform/

Table 2

Locus	pLOH	LOH	Add allele	Subst allele	Total
D8S1179	4	1	3	0	8
D21S11	7	2	1	0	10
D7S820	1	1	0	0	2
CSF1P0	1	6	0	0	7
D3S1358	5	1	1	0	7
TH01	4	0	1	0	5
D13S317	7	1	3	0	11
D16S539	1	3	0	0	4
D2S1338	1	9	2	0	12
D19S433	5	1	3	0	9
vWA	2	1	6	1	10
TPOX	2	2	0	0	4
D18S51	6	6	2	0	14
D5S818	13	2	0	0	15
FGA	7	3	1	0	11
AMEL.	8	3	0	0	11
TOT.	74	42	23	1	140

Isoamyl alcohol 25:24:1 and with 1 passage with Chloroform/Isoamyl alcohol 24:1. The DNA fraction was recovered in 100 μl of water and quantified by Eppendorf Biophotometer. The DNA was used in 1–3 ng aliquots in subsequent PCR amplifications.

DNA amplification was performed using the AmpF*l*STR® Identifiler™ PCR amplification kits (Applied Biosystems) [1]. The amplified alleles were separated by ABI PRISM 310 capillary electrophoresis (Applied Biosystems) using Genescan 500 LIZ size standard and allelic ladders to evaluate the sizes of the PCR products. The electrophoresis results were analysed using Genotyper version 2.0 software (Applied Biosystems) with a cut-off filter Kazam 20, which is generally used to exclude background noise when working with forensic samples of single origin.

Microsatellite instability in a carcinoma sample was identified by detection of abnormal alleles (allelic insertion) at the STR loci compared with the control tissue.

Loss of heterozygosity was identified by allelic loss (allelic deletion) in the tumour tissue as compared with the heterozygotic control sample. A sample was considered to be pLOH when a decreased fluorescence signal was observed in relation to the other allele.

3. Results and discussion

In our study we detected four kinds of changes between normal and tumour tissue: partial Loss of one allele (pLOH), complete Loss of one allele (LOH), occurrence of an additional allele and occurrence of a new allele instead of that found in normal tissue [2].

Only one-fourth of samples presented the same genotypes in Normal Fresh, Normal Fixed, Tumour Fresh and Tumour Fixed tissue specimens, the rest of samples had at least one altered locus.

Twenty percent of Normal Fixed Tissue specimens displayed allelic alterations; of these, 50% were pLOH, 25% were LOH and 25% consisted in additional allele (Table 1).

Sixty-four percent of Tumour Fresh Tissue specimens showed allelic alterations; of these, 72% were pLOH, 17% were LOH and 10% consisted in additional allele.

Seventy-two percent of Tumour Fixed Tissue specimens displayed allelic alterations; of these, 40% were pLOH, 45% were LOH, 14% consisted in additional allele, and 1% showed a substitution of a new allele instead of that found in normal tissue (Table 2).

The number of pLOH and LOH slightly exceeded the number of allelic addition or substitution. The lowest alteration frequencies were found at D7S820, D16S539 and TPOX. The most frequently altered loci were D5S818 and D18S51, according to Vauhkonen et al. [3].

The forensic marker D18S51 was located in the 18q chromosome, and contains also the gene DCC, which is often deleted in colorectal cancer.

References

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